

# 3.8.2015

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MONDAY, 8/3

Petra, Tamannae

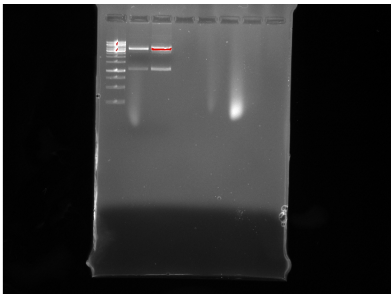
Did restriction of AH043 with SpeI and XbaI following mostly the protocol. However, the incubation time was 1,5 h and the restriction was done with 250 ng and 500 ng.

Made 1,3 % agarose gels with EtBr. Ran restrictions of AH043 in one gel (40 min, 120 V) and KAPA PCR products of 2nd VioA and 2nd BglX in another (25 min, 120 V). Pipeting order was for one: 1. ladder 2 ul 2. restriction of AH043 (250 ng) 3. restriction of AH043 (500 ng), and for another (double):

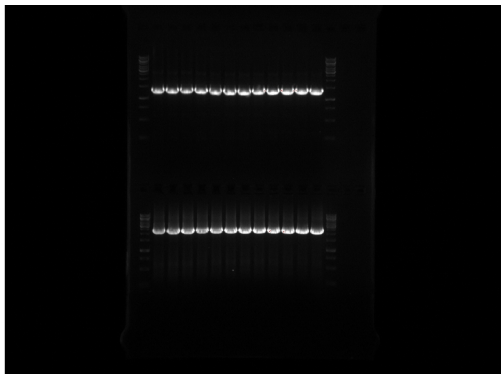
I 1. ladder 2ul 2.-13. gradient KAPA PCR of 2nd VioA 14. ladder 2 ul

II 1. ladder 2ul 2.-13. gradient KAPA PCR of 2nd BglX 14. ladder 2 ul

Geldoc\_2015-08-03\_14hr\_49min\_restrictionofAH043.jpg



Geldoc\_2015-08-03\_16hr\_14min\_2ndVioA\_2ndBglX.jpg



Did PCR purifications of 2nd VioA and 2nd BglX according to the kit protocol. Added 78,4 binding buffer to both purifications. NanoDrop result of the purifications:

Table1

Sample	DNA (ng/ul)	A260/A280
2nd VioA	328,6	1,86
2nd BglIX	345,9	1,83

Overlapping PCR for CAR construct

Reaction mix with backbone:

**5µl** 5 x Buffer

**0,75 µl** 10mM dNTP mix

**0,5 µl** KAPA HiFi HotStart DNA Polymerase

**3,35 ul** CAR Part 1

**0,62 ul** CAR Part 2

**1,98 ul** CAR Part 3

**12,8 ul (256 ng)** AH043 (a bit smaller than the amount listed on protocol): restriction mix concentration: 20 ug/ul

Reaction mix without backbone:

**12,8 µl** H<sub>2</sub>O (Calculate the amount of water based on how much DNA you use)

**5µl** 5 x Buffer

**0,75 µl** 10mM dNTP mix

**0,5 µl** KAPA HiFi HotStart DNA Polymerase

**3,35 ul** CAR Part 1

**0,62 ul** CAR Part 2

**1,98 ul** CAR Part 3

Ran PCR reaction for both of the mixes according to the protocol.

Transformed overlapping PCR (oe-PCR) reaction with backbone to TOP10. Followed the protocol. Plated transformants to ampicillin plates (50 ul and 200 ul) straight after adding SOC media.

Did restriction of AH043 with SpeI and XbaI following mostly the protocol. However, the incubation time was 1,5 h and the restriction was done with 1000 ng and 3000 ng. The restriction of AH043 is stored in the freezer.

Did o/n culture of AH043 in 2 ml LB with AMP.

# 4.8.2015

TUESDAY, 8/4

Did miniprep of yesterday's o/n culture of AH043 following the kit protocol.

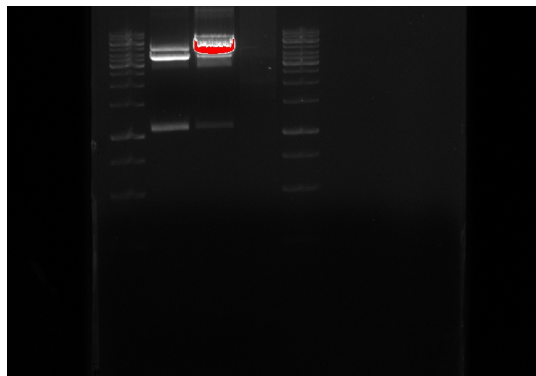
NanoDrop result of AH043:

Table1

Sample	DNA (ng/ul)	A260/A280
AH043	55,4	1,84

Made a 1,3 % agarose gel with EtBr to check yesterday's restrictions of AH043. Ran the restrictions on the gel for 50 min with 120 V. Pipeting order was 1. ladder 2 ul 2. restricted AH043 (1000 ng) 3. restricted AH043 (3000 ng)

Geldoc\_2015-08-04\_11hr\_52min\_AH043.jpg



Did gel purification of the restrictions of AH043 following the kit protocol.

NanoDrop result of the gel purification:

Table2

Sample	DNA (ng/ul)	A260/A280
AH043 1000 ng	9,1	1,85
AH043 3000 ng	3,6	1,86

Checking CAR over extension PCR plates (reaction with backbone, transformed yesterday).

- Growth on every plate
- All the colonies are red -> The original insert has gone back to the vector
- PCR Reaction with gel purified CAR over-extension PCR without backbone not successful

Did gel electrophoresis for CAR Over-extension PCR without backbone (0,625% Agarose gel, 110 V, 30 min).

Adding a terminator to the end of T7 promoter that our commercial backbones contain:

We don't want an extra T7 promoter to our plasmids since our constructs already contain the same promoter. We will add an extra terminator to block the promoter and to prevent the extra promoter from messing up the reading frame of our constructs.

Restricted plasmid backbones pACYC and pCDF duet 1 with EcoRI and PstI to add a terminator after the T7 promoter our backbones contain. Restricted the chosen terminator (AH006, BBa\_B0015) with the same restriction enzymes. Followed the restriction digestion protocol. Note: pACYC and AH006 have chloramphenicol resistance -> we have to screen the colonies with the extra terminators using colony PCR.

Restricted AH043 backbone XbaI and SpeI to do a test Gibson assembly tomorrow. Added 500 µl DNA to get a suitable final concentration (500 ng / 25 µl = 20 ng/µl).

Followed the protocol. Incubated 75 min on 37 °C before inactivation.

Stored the reaction to freezer.

KAPA PCR for CAR construct (linear construct for oe-PCR): 4987 bp

Used the remaining 20 µl reaction mix from oe PCR.

Made an additional MasterMix (25µl) without template DNA:

**17,25 µl** H<sub>2</sub>O (Calculate the amount of water based on how much DNA you use)

**5 µl** 5 x Buffer

**0,75 µl** 10mM dNTP mix

**0,75 µl** 10µM forward primer

**0,75 µl** 10µM reverse primer

**0,5 µl** KAPA HiFi HotStart DNA Polymerase

Mixed Mastermix with oe-PCR reaction mix -> 45 µl PCR sample.

95° C - 3 min

98° C - 30 sec

64° C - 30 sec

72° C - 5 min

72° C - 5 min

4° C - forever

Repeated underlined cycles 25 times

# 5.8.2015

WEDNESDAY, 8/5

Petra

Gibson assembly for CAR

Table1

Constru ct	Length (bp)	Concentration (ng/ul)	DNA (ng) needed for 0.05 pmol	DNA (ul) needed for 0,05 pmol
CAR part 1	1945	53,7	60,1	1,1
CAR part 2	1500	224,7	46,35	0,2
CAR part 3	1542	70,8	47,64	0,7
AH043	4031	20	124,5	6,2
Total				8,2

Water: 1,8 ul

Pipetted DNA (AH043, CAR part 1, Fixed CAR part 2, CAR part 3) to 1,5 ul tube according to Table 1. Added 1,8 ul water. Added 10 ul NEBuilder HiFi DNA Assembly MasterMix. Incubated the mix in 50 C for 60 minutes. Used 2 ul for transformation.

ELIC cloning for CAR

Table2

Construct	Lenght	Concentration (ng/ul)	f m ol D N A n e e d e d f o r E L I C	DNA (ng)	D N A ( u l)
CAR part 1	1945	53,7	4 8, 1 8	57,91	1 , 1
fixed CAR part 2	1500	224,7	4 8, 1 8	44,66	0 , 2
CAR part 3	1542	70,8	4 8, 1 8	45,91	0 , 6
AH043	4031	20	1 6, 0 6	40	2
Total					3 , 9

Water: 6,1 ul

Pipetted DNA (AH043, CAR part 1, Fixed CAR part 2, CAR part 3) to 1,5 ul tube according to Table 2. Added 6,1 ul water. Total reaction volume for ELIC was 10 ul. Incubated Reaction mix in room temperature for 60 minutes. When doing transformation, added competent cells straight to the same tube where reaction mix was.

Transformation of Gibson and ELIC reactions into TOP10.

Followed the protocol. Added 250 ul SOC after heat shock. Before plating the tubes sat on ice for 45 minutes. Plated 50 ul and 200 ul of each reaction on ampicillin plates (4 plates in total) and left to 37 C incubator overnight.

Made a 1,3 % agarose gel with ETBR. Used a thin comb.

Ran 5 ul samples with 1 ul LD on gel:

- PCR reaction of CAR from oe-PCR (ran o/n)
- AH043 backbone (restricted yesterday).

Used Gene O'Ruler 1 kb ladder. Ran the gel for 30 min, 120V.

EDITED\_CAR\_oe-PCR\_AH034\_restriction\_Geldoc\_2015-08-05\_11hr\_43min.jpg



Gel results can be seen from the picture above. CAR pcr reaction has been successful as well as oe-PCR reaction done before to create CAR construct: 4987 kb DNA piece can be seen. Restriction for AH043 has been successful as well according to the gel.

Amphiphilic DNA (amph) arrived from Invitrogen. Spinned the tube, added 50 ul sterilized water and incubated in room temperature for 1h.

- Final concentration: 100 ng/ul
- Made an aliquot for PCR: 1 ul DNA to 9 ul water -> 10 ng/ul

Purified CAR PCR reaction (from oe-PCR) with GeneJET PCR Purification kit:

- Followed the kit protocol
- Final elution 25 ul
- Nanodrop results: 50,3 ng/ul, A260/A280: 1,81
- Naming: CAR oe-PCR

Made a 10 ng/ul dilution of CAR oe-PCR

- 1 ng CAR oe-PCR DNA (~50 ng/ul)
- 5 ul sterilized water

Made a 1 ng/ul dilution of CAR oe-PCR for PCR

- 1 ng CAR oe-PCR dilution (10 ng/ul)
- 9 ul sterilized water

Gradient PCR for amph (WITH linker):

Made a template DNA dilution

- 1 ul 10 ng/ul amph aliquot to 9 ul sterilized water

13x reaction mix (325 ul):

**217,75 ul** sterilized water

**65 ul** 5x buffer

**9,75 ul** 10mM dNTP mix

**9,75 ul** 10μM P044

**9,75 ul** 10μM P045

**6,5 ul** KAPA HiFi HotStart DNA Polymerase

**6,5 ul** fixed Amph DNA dilution (1 ng/ul)

95° C - 3 min

98° C - 20 sec

56,5-71,5° C - 20 sec

72° C - 1 min

72° C - 3 min

4° C - forever

Repeated underlined cycles 25 times

KAPA PCR for CAR from oe-PCR

**16,75 μl** H<sub>2</sub>O (Calculate the amount of water based on how much DNA you use)

**5µl** 5 x Buffer  
**0,75 µl** 10mM dNTP mix  
**0,75 µl** 10µM forward primer  
**0,75 µl** 10µM reverse primer  
**0,5 µl** KAPA HiFi HotStart DNA Polymerase  
**0,5 ul** CAR oe-PCR 1 ng/ul dilution

95° C - 3 min

98° C - 30 sec

65° C - 30 sec

72° C - 5 min

72° C - 5 min

4° C - forever

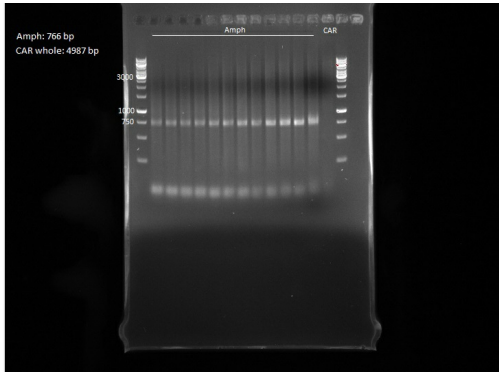
Repeated underlined cycles 25 times



# 6.8.2015

THURSDAY, 8/6

EDITED\_GPCR\_Amph\_and\_CAR\_oe-PCR\_Geldoc\_2015-08-06\_11hr\_16min.jpg



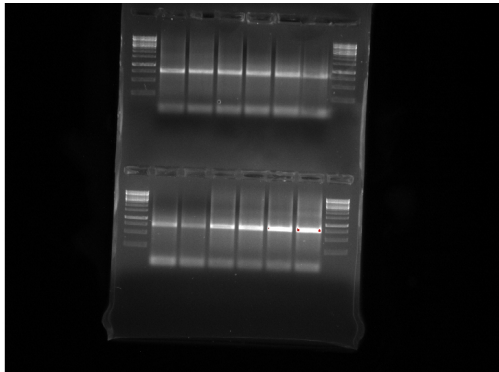
Made a 1,3 % gel with ETBR. Ran 5 ul samples with 1 ul LD of all the PCR reactions ran overnight (Amph gradient and CAR oe-PCR). Ran the gel 30 min, 120 V. Results can be seen in the picture above.

- Amph gradient was successful, continued with gel purification
- CAR oe-PCR failed

Purifying AMPH:

Made a 1,3 % gel with ETBR. Used two big combs. Pipetted 4 ul LD to all the 12 Amph PCR reactions. Ran the reactions on gel: 20 min, 120 V.

Geldoc\_2015-08-06\_15hr\_17min\_Amph\_gel\_purification.jpg



Cut Amph DNA out and purified it with Gene JET gel extraction kit. Followed the protocol.

- Nanodrop results: 99,6 ng/ul, A260/A280: 1,12
- The DNA is not pure, not sure if it can be used later
- Stored to freezer, name: Amph + linker

Checked transformation plates from yesterday

- CAR Gibson: 7 colorless colonies among the red colonies -> continued with colony PCR
- ELIC: some colorless colonies among the red colonies -> colony PCR later
- Both reactions produced potential colonies for screening: those colonies that aren't red don't contain red color producing insert. Instead they might contain CAR construct, so screening is needed

KAPA Colony PCR for CAR to screen the potential colonies:

Choose seven white colonies from Gibson plates. Treated the colonies according to colony PCR protocol. Plated all the seven colonies to a fresh ampicillin plate.

Reaction mix (25  $\mu$ l):

**15,25  $\mu$ l** H<sub>2</sub>O

**5  $\mu$ l** 5 x Buffer

**0,75  $\mu$ l** 10mM dNTP mix

**0,75  $\mu$ l** 10 $\mu$ M forward primer

**0,75  $\mu$ l** 10 $\mu$ M reverse primer

**0,5  $\mu$ l** KAPA HiFi HotStart DNA Polymerase

**2  $\mu$ l** template DNA from the chosen colonies

Made an own reaction mix for all the seven colonies.

KAPA colony PCR for CAR from Gibson assembly: PCR program

95° C - 3 min

98° C - 30 sec

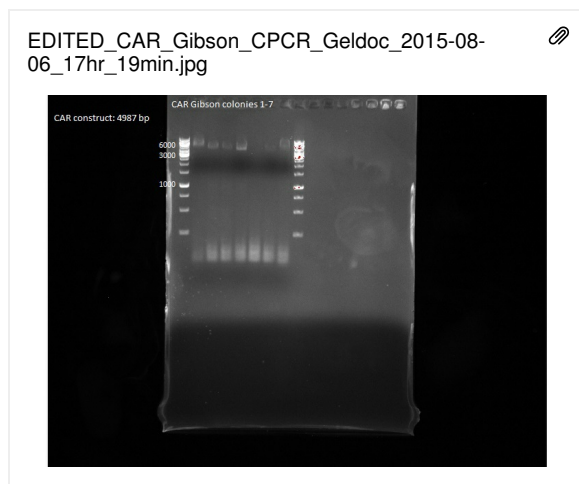
64° C - 20 sec

72° C - 5 min

72° C - 5 min

4° C - forever

Repeated underlined cycles 25 times



Checking CAR Gibson colony PCR results:

Made a 1,3 % gel with ETBR. Ran 5  $\mu$ l samples with 1  $\mu$ l LD of all the seven PCR reactions. Ran the gel 30 min, 120 V. Results can be seen in the picture above:

- 6 from 7 colonies produced a band that is somewhat the size we want
- Decided to make o/n cultures of them all and decide tomorrow which ones to send to sequencing

o/n cultures of CAR Gibson colonies:

- 2  $\mu$ l ampicillin stock to 2 ml LB
- incubation with shaking in 37 C

Made o/n culture of TOP10 to make more competent cells tomorrow:

- x  $\mu$ l streptomycin to 2 ml LB
- incubation with shaking in 37 C

Did KAPA PCR reaction of GFP.

GFP reaction mix (325  $\mu$ l):

217,75  $\mu$ l sterilized water

65  $\mu$ l 5x buffer

9,75  $\mu$ l 10mM dNTP mix

9,75  $\mu$ l 10 $\mu$ M P028

9,75  $\mu$ l 10 $\mu$ M P029

6,5  $\mu$ l KAPA HiFi HotStart DNA Polymerase

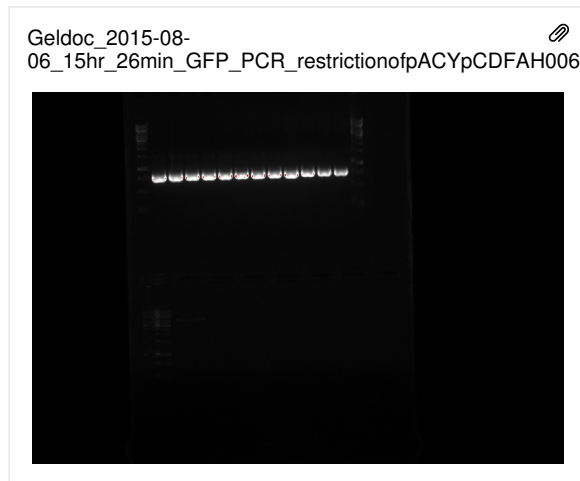
6,5 ng Interlab Measurement Device 4 (D4)

The PCR program for GFP:

95°C - 3 min  
98°C - 20 sec  
54,7°C-69,2°C -15 sec  
72°C - 50 sec  
 72°C - 1,5 min  
 4°C - forever  
 Repeated underlined cycles 25 times

Made a 1,3 % agarose gel with EtBr. Run PCR product of GFP and restrictions of pACYC, pCDF-Duet1 and AH006 in the gel for 25 min with 120 V. Pipeting order was:

I 1. ladder 2 ul 2.-13. gradient KAPA PCR of GFP 6 ul 14. ladder 2 ul  
 II 1. ladder 2 ul 2. pACYC 6 ul 3. pCDF-Duet1 6 ul 4. AH006 1,2 ul



Did PCR purification of GFP according to the kit protocol. Added 77,6 ul binding buffer.  
 NanoDrop result was:

Table1

Sample	DNA (ng/ul)	A260/A280
GFP	235,9	1,84

Ligated restricted AH006 to restricted pACYC and to restricted pCDF-Duet1 following mostly the T4 ligase protocol. However, overall mix was 20,2 ul, which is 0,2 ul over the recommended amount.

Ligation mix:

12 ul AH006  
 6 ul pACYC/pCDF-Duet1  
 2 ul Buffer  
 0,2 ul T4 ligase

Transformed ligations of pACYC (with AH006) and pCDF Duet1 (with AH006) to Top10 following the protocol. Plated 100 ul pACYC to a CAM plate and 50 ul and 150 ul pCDF-Duet1 to SM plates.

# 7.8.2015

FRIDAY, 8/7

Petra, Tamannae

Making chemically competent cells:

Followed the protocol. Started incubating refreshed culture at 7.30.

Cloramphenicol plates:

Followed the protocol. Added 500 ul chloramphenicol stock (34 ug/ul final concentration)

Minipreped CAR o/n cultures (from colonies 2,3,4 and 7) for sequencing. Used NucleoSpin plasmid EasyPure kit. Followed the protocol.

Nanodrop results:

Table1

Construct	Concentration (ng/ul)	A268/A280
CAR 2	97,6	1,93
CAR 3	112,0	1,90
CAR 4	119,4	1,88
CAR 7	94,4	

Started testing the strain E. coli BL21(DE3) $\Delta$ yjgB  $\Delta$ yqhD and its propane production capacity. Did the overnight culture with 21,5 mL of LB, with incubation at 38 °C and 230 rpm. Inoculated with 8,4 % (v/v) of the previous culture by adding 255,5 ml (flasks with 500ml volume) of TB media containing appropriate antibiotics (amp 100 ug/ml + spec 50 ug/ml + str 20 ug/ml + chloramphenicol 34  $\mu$ g/ml). Incubated 3,5 h at 38 °C and 180 rpm until OD600 was 0,5192. Because of the OD samples taken out, the volume of cultivation was 253 ml at this point. Added 60,1  $\mu$ l IPTG (500 mg/ml) for the concentrations of 0,5 mM and induced the culture with 2 h incubation at 30 °C for 2 h instead of 4 h because of the time limit. Pelleted the cells with centrifuge (3214xg, 4 21 °C) and resuspended with 63,25 ml of TB-media. Transferred 8 ml of culture for each 22 ml GC-vials with a gas tight syringe and started analysis. Measured three samples with GC but no marks from propane detected. More information available at <http://2015.igem.org/Team:Aalto-Helsinki/Results>.

Did KAPA gradient PCR reactions of amph NOTERM, amph + prefix + suffix without linker and AtoB part 2 (AtoB2).

amph NOTERM 13x reaction mix (325 ul):

217,75 ul sterilized water

65 ul 5x buffer

9,75 ul 10mM dNTP mix

9,75 ul 10 $\mu$ M P046

9,75 ul 10 $\mu$ M P051

6,5 ul KAPA HiFi HotStart DNA Polymerase

6,5 ng Amph

amph + term + prefix + suffix without linker 13x reaction mix (325 ul):

217,75 ul sterilized water

65 ul 5x buffer

9,75 ul 10mM dNTP mix

9,75 ul 10 $\mu$ M P045

9,75 ul 10 $\mu$ M P046

6,5 ul KAPA HiFi HotStart DNA Polymerase  
6,5 ng Amph

AtoB2 13x reaction mix (325 ul):  
217,75 ul sterilized water  
65 ul 5x buffer  
9,75 ul 10mM dNTP mix  
9,75 ul 10µM P011  
9,75 ul 10µM P033  
6,5 ul KAPA HiFi HotStart DNA Polymerase  
6,5 ng AtoB part 2

amph NOTERM and amph + prefix + suffix without linker had same PCR program:

95°C - 3 min  
98°C - 20 sec  
55,5°C-70,5°C -15 sec  
72°C - 45 sec  
72°C - 1,5 min  
4°C - forever  
Repeated underlined cycles 25 times

The PCR program for AtoB2 was:

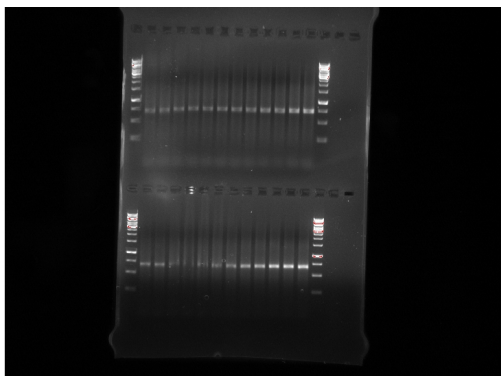
95°C - 3 min  
98°C - 20 sec  
55,5°C-70,5°C -15 sec  
72°C - 120 sec  
72°C - 1,5 min  
4°C - forever  
Repeated underlined cycles 25 times

Made a 1,3 % agarose gel with EtBr. Ran the PCR products of amph NOTERM and amph + prefix + suffix without linker in the gel for 25 min with 120 V.

Pipeting order was (double):

I 1. ladder 2 ul 2.-13. gradient amph NOTERM 6 ul 14. ladder 2 ul  
II 1. ladder 2 ul 2.-13. gradient amph + prefix + suffix without linker 6 ul 14. ladder 2 ul

Geldoc\_2015-08-  
07\_15hr\_47min\_nolinker\_and\_no\_TER\_amph.jpg



Stored the PCR products of AtoB2, amph NOTERM and amph + prefix + suffix without linker in the fridge.